from the mitochondrial interior. Currently structural studies of various Bax-lipid assemblies are ongoing.

Protein-Lipid Interactions II

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Dynamic Studies of the Tumour Suppressor Protien PTEN Binding to Membranes Composed PI(4,5)P2 and Various Anionic Lipids

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The tumor suppressor gene, *Phosphatase and tensin homolog deleted on chromosome 10* (PTEN) encodes a protein that is involved in many human cancers. It binds to the plasma membrane to dephosphorylate the 3-position of phosphatidylinositol-3,4,5-trisphosphate producing phosphatidylinositol-4,5-bisphosphate (PI[4,5]P_2). PTEN is an agonist to PI 3-kinase, thereby inhibiting the PI3K/Akt signaling pathway and controlling cell proliferation and survival. PTEN membrane association depends strongly on the composition and lateral distribution of the lipids in the membrane. Using stopped flow kinetic and steady state fluorescence experiments, we characterize the different steps associated with PTEN membrane association and dissociation.

We find that PTEN membrane association is governed by the nature of the anionic lipids in the model membrane and their lateral distribution. It has been found previously that PTEN interacts synergistically with phosphatidylserine (PS) and phosphatidylinositol-4,5-bisphosphate (PI(4,5)P_2). When comparing the kinetics of PTEN binding to PC/PS (30%) and PC/PI(4,5)P_2 (5%) vesicles, we find a faster k_{on} value and slower k_{off} rate constant for the PI(4,5)P_2 containing vesicles. When both PS and PI(4,5)P_2 are present in the vesicles, we find the fastest association rate constants. In model membrane systems, PS and PI(4,5)P_2 show only limited co-localization. Therefore, the question arises whether PS in biological membranes is the second binding partner, in addition to PI(4,5)P_2, of PTEN. We hypothesize that phosphatidylinositol (PI), which forms domains with PI(4,5)P_2, may replace PS in at least some physiological scenarios as the second binding partner. We find that PTEN binding to PI vesicles is as strong as PTEN binding to PS vesicles, confirming the hypothesis that PTEN binding to PS or PI is non-specific electrostatic. This finding is a major paradigm shift in the PTEN field.

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Computational Modeling of the N-Terminus of the Human Dopamine Transporter (hDAT)

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DAT is a transmembrane protein in the family of Neurotransmitter:Sodium Symporters (NSS). The NSS are responsible for the clearance of neurotransmitters from the synaptic cleft, and for their translocation back into the presynaptic nerve terminal. In contrast to its bacterial homologue, the leucine transporter (LeuT), mammalian DAT contains long intracellular N- and C-terminal domains that are strongly implicated in the transporter function. The N-terminus (N-term), in particular, regulates the process of efflux, i.e. the reverse transport of the substrate (dopamine) through DAT. Currently, the molecular mechanisms of the efflux remain largely elusive due to lack of structural information on the N-terminal segment. To seek a valid prediction of the three-dimensional (3D) fold of the N-terminus from the human DAT (hDAT) we used a combination of ab initio structure prediction tools and extensive atomistic MD simulations. The ab initio modeling revealed structured regions within the first 57 residues of the N-term. The subsequent long (2.2 μs in total) atomistic MD simulations of the N-terminal in complex with a lipid membrane showed that the identified secondary structure elements were stable on the simulation timescales and were involved in specific interactions with pertinent models of the cell membrane. The N-term engages with lipid membranes through direct electrostatic interactions with the charged lipids PIP2 (phosphatidylinositol 4,5-biphosphate) and PS (phosphatidylserine). We identify specific motifs along the N-terminal domain that are implicated in such interactions and show that differential modes of N-term/membrane associations result in differential positioning of the structured segments on the membrane surface. The molecular insights we report regarding the preferred modes of interaction of the DAT N-term with model membranes provide a novel structural context for future explorations of the mechanistic questions related to the functional mechanisms of this transporter.

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Localization of Lipids to the Cavity and Transmembrane Domain of ATP-Binding Cassette Transporter ABCB10, as Revealed by Molecular Dynamics Simulations

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ABCB10 is a poorly understood ATP-binding cassette transporter of the human inner mitochondrial membrane (IMM). The IMM is characterized by the presence of cardiolipin (CDL), a negatively-charged phospholipid that has been shown to increase protein stability and function upon binding. After PC and PE lipids, CDL contributes the greatest by mass to the IMM composition. To identify binding domains for these lipids on ABCB10, we performed coarse-grained molecular dynamics simulations of ABCB10 embedded in a nanodisc with a CDL-POPC-POPE lipid bilayer. We found that the binding sites for each lipid are distinct on the transmembrane region, with POPE and CDL exhibiting a leaflet-dependent binding preference for the transporter. We also demonstrate that lipid and water entry into the ABCB10 cavity occur in the closed conformation, suggesting that the as yet unidentified ABCB10 substrate is likely a lipid.

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Investigating Sec14 Domain Lipid Binding using Structural Modeling

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Interactions between proteins and lipids play important roles in cellular processes, including signaling and growth, and a detailed molecular understanding of protein-lipid binding partners can improve our understanding of these processes. The widely occurring Sec14 family of proteins is known to bind phospholipids. While the family's lipid binding domain is highly conserved across species, each protein has a unique binding specificity profile. Being able to predict this profile based on the sequence of a particular Sec14 domain would be useful in understanding the physiological roles of these proteins. To this end, we used a combination of homology modeling and molecular dynamics simulations to develop a structural model of the Patellin1 (PATL1) Sec14 domain. This model provided a structural explanation for the previously measured preference of PATL1 for phosphatidylinositol-5-phosphate lipids. The PATL1 model was further evaluated through in vitro lipid binding measurements. Observations from the PATL1 lipid binding model were used to predict the lipid binding profile of yeast Sec14 which was also confirmed by in vitro lipid binding. Thus, structural modeling of Sec14 domains can be used to predict the lipid binding of Sec14 domain containing proteins, leading to hypotheses about the in vivo localization and possible functions of these proteins. This structural information will also be useful in efforts to engineer Sec14 domains with desired lipid binding specificity.

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A Microfluidic Device to Study Translocation Across Lipid Membranes

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The regulation of protein trafficking across cellular membranes, done by transmembrane proteins, is a vital process known as translocation. However, protein translocation is not completely understood and traditional electrophysiology techniques cannot give an insight into the mechanical properties of translocation. On the other hand, optical tweezers has been shown to be a suitable candidate to study the translocation of soluble proteins but becomes a challenge with transmembrane proteins. Hence there is a need to develop a biophysical tool that allows the study of transmembrane proteins into their membrane environment with optical tweezers.

There is an increasing effort to miniaturize black lipid membranes (BLM), and several approaches have now been developed to study the translocation of proteins in miniaturized systems. In this work, we develop a microfluidic system suitable for the study of transmembrane proteins into artificial membranes. The advantages of our approach include: real-time control over the charge gradient across the membrane, dynamic exchange of buffers, and capability to combine with force-spectroscopy techniques.

We demonstrate the formation of a free-standing BLM on a glass micro-device by measures of capacitance and electron flux detection through toxin pores. This microfluidic device is combined with a high-resolution optical tweezers for the study of protein translocation across membranes and the evaluation of current translocation models.